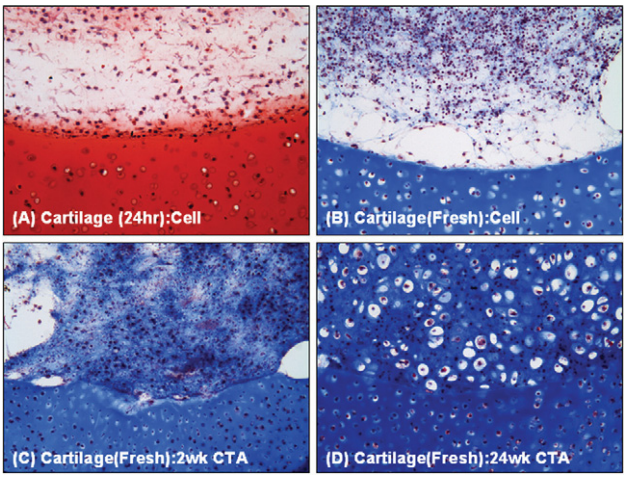

Figure 1. Histological analysis stained with (A) Saf-O and (B–D) Alcian Blue: (A) 4 w growth; preconditioned (24 h) defect:cells, (B) 6 w growth; fresh defect:cells, (C) 4 wk growth; fresh defect:2 wk CTA, and (D) 6 w growth; fresh defect:24 wk CTA.)

out scaffold or foreign material. Good integration occurred with our CTA. More mature CTA were more successful at filling the defect and showed a uniform PG rich matrix. Biomechanical tests are underway to assess the mechanical integrity of the interface. These CTAs presents a new simplistic approach to cartilage repair and with additional influences such as force and specialized growth conditions these CTA may prove to yield a clinically relevant repair platform.

484

INHIBITORY EFFECT OF SOCS-3 AND INDISPENSABLE ROLE OF NF-KB IN CHONDROGENESIS OF HUMAN MESENCHYMAL STEM CELLS

G.T. Heldens, E.L. Vitters, O.J. Arntz, S. Veenbergen, M.B. Bennink, F.A. van de Loo, W.B. van den Berg, P.M. van der Kraan
Experimental Rheumatology and Advanced Therapeutics, Nijmegen Ctr. for Molecular Life Sci., Univ. Med. Ctr. Nijmegen, Nijmegen, Netherlands

Purpose: Articular cartilage shows poor intrinsic repair, leading to progressive joint damage. Therapies like marrow stimulation or tissue engineering of cartilage depend on the chondrogenic differentiation of progenitor cells. However, this chondrogenesis needs to take place in a diseased joint with an altered environment. In this environment inflammatory mediators will be present in elevated levels and these will activate inflammatory pathways in differentiating chondrocyte precursors. We examined the role of two inflammatory pathways in chondrocyte differentiation, the NF-kB and SOCS-3 pathway as possible therapeutic targets to optimize cartilage regeneration.
Methods: Human mesenchymal stem cells (hMSC) (Lonza) were differentiated into the chondrogenic lineage. Cells were pelleted in serum free chondrogenic medium supplemented with TGF-beta and BMP-2. Interleukin-1 (IL-1) and osteoarthritic synovium derived conditioned medium, both a model for inflammatory stimulation, were added to hMSC undergoing chondrogenic differentiation.
Two downstream pathways known to be induced by catabolic factors such as IL-1 were examined: the NF-kB and the SOCS-3 pathway. To study the specific role of the NF-kB pathway cells were lentivirally transfected with P65 siRNA hairpin to block P65 expression. Activation of the SOCS-3 pathway was mimicked by lentiviral overexpression of SOCS-3. Chondrogenesis was examined by determining mRNA levels of chondrogenic markers COL2A1 (collagen type II) and ACAN (aggrecan). Proteoglycan deposition was analyzed by safranin O staining of histological sections.
Results: Chondrogenic differentiation, as measured by expression of type II collagen and aggrecan and safranin O proteoglycan staining, was induced in the hMSC already strongly at two weeks under the described anabolic conditions. IL-1 as well as osteoarthritis synovium-conditioned medium inhibited chondrogenesis of hMSC. Conditioned medium induced SOCS-3 mRNA strongly in the hMSC undergoing chondrogenesis. IL-1 also induced SOCS-3 mRNA, albeit at a lower level. Lentiviral overexpression of SOCS-3 lead to total inhibition of chondrogenesis already in the condition without catabolic factors. In addition, blocking the NF-kB pathway P65 siRNA resulted in complete inhibition of chondrogenesis, even under anabolic conditions. Indicating that NF-kB signaling is required for normal chondrogenesis
Conclusions: Our study shows that the NF-kB pathway appears to be indispensable for chondrogenesis. This demonstrates NF-kB is unsuitable as a target to stimulate chondrogenesis in an inflammatory milieu. SOCS-3 was induced by catabolic factors and overexpression of SOCS-3 potently inhibited chondrogenesis. SOCS-3 is therefore a potential candidate target to modulate the inhibiting effect of catabolic factors on chondrogenesis and blocking SOCS-3 in diseased joints could improve cartilage repair through de-repression of chondrogenesis.

485

IN VITRO GENE KNOCKDOWN OF *SOX9* IN RAT MSC AFFECTS CELL CYCLE PROGRESSION

S. Stoeckl[1,2], C. Goettl[1,2], J. Grifka[2], S. Graessel[1,2]
[1]Ctr. for Med. Biotechnology, Regensburg, Germany; [2]Dept. of Orthopaedic Surgery, Regensburg, Germany

Purpose: The transcription factor *Sox9* plays an important role in the regulation of skeletal growth and cartilage formation. *De novo* mutations in the coding region of *Sox9* cause campomelic dysplasia, an autosomal dominant, frequently lethal disease. Moreover, *Sox9* is known to control the initial steps of chondrogenic differentiation in mesenchymal stem cells (MSC). Recent studies have emphasized the role of cell cycle regulators like p21 in the coordination of chondrocyte proliferation and differentiation. But otherwise, less is known about the function of the chondrocyte transcription factor *Sox9* in cell cycle progression and cellular distribution in the different cell cycle phases. There is evidence that *Sox9* might alter cell cycle progression to facilitate its pro-differentiating function in a rat chondrocytic cell line.
Methods: We have employed RNA interference (RNAi) to study the effects of *Sox9* gene knockdown *in vitro*, in undifferentiated multipotent rMSC (rat mesenchymal stem cells derived from bone marrow). Cells were transduced with a retroviral vector containing a *Sox9*-specific shRNA. After selection with the appropriate antibiotics, the knockdown level of *Sox9* was determined by gene expression analysis with quantitative PCR and the protein amount by western blotting. Subsequently, a real-time PCR based cell cycle profiler pathway array was performed to detect alterations in the expression of 84 different cell cycle specific genes after an inhibiting *Sox9* expression in rMSC. For further analysis of the cell cycle distribution, cells were fixed with 70% ethanol and stained with propidium iodide to investigate cell populations for G_0/G_1, S and M phases by flow cytometric measurement.
Results: A reproducible knockdown, on average between 50% and 90%, was observed in rMSC on the mRNA and the protein level. Cells expressing less *Sox9* caused by the *Sox9*-shRNA, showed a clear decrease in proliferation. Hence, there is evidence for an effect of *Sox9* on cell cycle progression. The *Sox9* gene